



Bioactive profile and anti-UV activities of ethyl acetate extract of *Rhizophora apiculata* leaves

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ABSTRACT

Mangroves plants such as *Rhizophora apiculata* are known to possess a diverse array of bioactive compounds with antioxidant and photoprotective properties. This study aimed to evaluate the anti-UV activity and bioactive composition of the ethyl acetate extract of *R. apiculata* leaves. The extraction was performed using the maceration method over 3 × 24 hours with ethyl acetate as the solvent. UV absorption was assessed using UV-Visible spectrophotometry, while compound identification was carried out through Gas Chromatography–Mass Spectrometry (GC-MS). The extract exhibited significant UV absorption in the 290–320 nm range, with Sun Protection Factor (SPF) values ranging from 2.530 ± 0.03 (low protection) to 37.080 ± 0.47 (high protection), indicating strong potential as a natural sunscreen agent. GC-MS analysis revealed several bioactive constituents, including lupeol, friedelan-3-one, cyclohexane-1,3,5-triphenyl, α-tocopherol, and 1,3,5,7-cyclooctatetraene—compounds recognized for their UV-absorbing and antioxidant activities. The synergistic action of these phytochemicals is believed to contribute to the extract's overall photoprotective efficacy.

Introduction

Protection against ultraviolet (UV) radiation is essential to prevent serious health risks. According to the American Cancer Society (ACS), approximately 100,000 cases of skin cancer are diagnosed annually in the United States alone. Furthermore, the World Health Organization (WHO) estimates that by 2040, UV radiation exposure will contribute to around 466,900 new cases of melanoma globally (Mota *et al.*, 2019).

The harmful effects of ultraviolet (UV) radiation on human health including skin damage, cataract formation, diabetes, immunological dysfunction, oxidative stress, DNA damage, photoaging, and an increased risk of skin cancer have intensified the demand for effective photoprotective agents. (Ahmed *et al.*, 2020; Bernard *et al.*, 2019, Kamari *et al.*,

2019; Lee *et al.*, 2021) While synthetic sunscreens dominate the market, concerns about their potential adverse effects on human health and the environment have spurred interest in natural alternatives (Chatzigianni *et al.*, 2022). Natural products derived from plants are gaining increasing interest as sustainable and effective photoprotective agents, aimed at reducing the reliance on synthetic sunscreen components (Saewan and Jimtaisong, 2015). Plants, with their rich reservoirs of bioactive compounds like phenolic, tannin, flavonoid, and alkaloid, which provide protection against UV-induced oxidative stress (Elbouzidi, *et al.*, 2025; Marchev *et al.*, 2020; Agati and Tattini, M 2010; Akbar and Hasan, 2024). These compounds can absorb harmful UV radiation, neutralize ROS, and support cellular defense mechanisms (Boo, 2020;

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Dunaway et al., 2018; Akbar et al., 2023). Among various plant sources, mangroves have emerged as a unique group with high pharmacological potential, owing to their adaptability to harsh environmental conditions and their rich phytochemical profiles. These include alkaloids, flavonoids, carotenoids, fatty acids, aliphatic alcohols, phenolic compounds, steroids, saponins, tannins, and terpenoids (Dahibhate et al., 2018; Mitra et al., 2021).

Mangrove plants are known for their diverse array of secondary metabolites that exhibit various pharmacological effects, including antioxidant, antibacterial, antimicrobial, anti-inflammatory, and antipyretic activities. (Abdel et al., 2016; Kalasuba et al., 2023; Surya et al., 2023). The mangrove species *Rhizophora* sp. is distributed globally, particularly along the coasts of tropical and subtropical regions, including Indonesia (Hamzah et al., 2018). Among these, *Rhizophora apiculata* is one of the most widely distributed species and is notable for its medicinal potential and diverse bioactivities, including antimicrobial, antioxidant, and anticancer properties. (Ramalingam & Rajaram et al., 2018). Meanwhile, the potential of *Rhizophora apiculata* for UV protection and the specific chemical constituents responsible for its anti-UV properties remain underexplored. According to (Ebrahimzadeh et al., 2014; Rahmasari et al., 2021) phenolic compounds and antioxidant activity play a critical role in protecting against photo-induced free radical reactions.

This study aims to evaluate the anti-UV activity of the ethyl acetate extract of *Rhizophora apiculata* leaves and to identify its phytochemical constituents through GC-MS analysis. By integrating bioactivity assessment with chemical profiling, this research seeks to explore the potential of this mangrove species as a natural and sustainable source for photoprotective agents.

Materials and Methods

Location and time of research

This research was conducted from June to August 2023. The sampling of *Rhizophora apiculata* mangrove leaves was carried out in the Alue Naga mangrove area, Syiah Kuala sub-district, Banda Aceh City.

Materials

The tools used in this research included a UV-Vis Spectrophotometer (Thermo Orion AquaMate 8000), quartz cuvettes, an evaporator, a blender/chopper, an oven, sample bottles, Whatman filter paper, a sieve, digital scales, beakers, Erlenmeyer flasks, test tubes, spatulas, and aluminum foil.

The materials used in this study consisted of *Rhizophora apiculata* leaf samples, ethyl acetate, ethanol, distilled water, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, and phenyl methyl siloxane.

Extraction

A total of 100 grams of *Rhizophora apiculata* leaves were macerated with 300 mL of ethyl acetate in a 1:3 (w/v) ratio over three consecutive 24-hour periods in a sealed container. Following maceration, the mixture was filtered, and the resulting filtrate was concentrated using a rotary evaporator at 40 °C to obtain the crude extract. The extract was then subjected to GC-MS analysis and evaluated for its sunscreen activity (Sormin et al., 2021).

Total phenolic assay

The total phenolic content was determined using the Folin–Ciocalteu assay. A 1 mL aliquot of the concentrated extract was mixed with 1 mL of ethanol and 5 mL of distilled water. Subsequently, 0.5 mL of 50% Folin–Ciocalteu reagent was added to the mixture, which was then homogenized and allowed to stand for 5 minutes. After that, 1 mL of 5% Na_2CO_3 solution was added, and the mixture was incubated in the dark for 60 minutes. Gallic acid was used as the standard for calibration. The total phenolic content was measured using a UV-Vis spectrophotometer at 725 nm, and the absorbance values were converted to total phenolic content, expressed as milligrams of gallic acid equivalent (mg GAE) per gram of sample (Febriani et al., 2020).

GC-MS Analysis

The bioactive profile of *Rhizophora apiculata* leaves extract was analyzed using gas chromatography-mass spectrometry (GC-MS). A 1 μL aliquot of the extract was injected into the GC-MS system for analysis. Chromatographic separation was carried out using an Agilent 19091S-433 HP-5MS column (5% phenyl methyl siloxane) with dimensions of 30 μm in length, 250 μm in diameter, and a film thickness of 0.25 μm . The oven temperature was programmed to increase from 100°C to 220°C at a rate of 15°C per minute, maintaining a constant flow rate of 1.0 mL/min. Helium was used as the carrier gas at a pressure of 10.5 psi, with a total flow rate of 140 mL/min and a split ratio of 1:50. The eluted compounds were detected using a mass spectrometer and identified by comparing their spectral profiles with reference data from a mass spectral database (NIST and WILEY libraries).

Sunscreen Activity

The sunscreen activity of mangrove leaf extract was evaluated using spectrophotometry by measuring the absorbance of the ethyl acetate extract at wavelengths between 290 and 320 nm. The anti-UV activity was determined based on the Sun Protection Factor (SPF), which reflects the extract's level of photoprotection. SPF values were calculated from the absorbance of extract samples dissolved in ethyl acetate at concentrations of 25, 50, 100, 200, 500, and 1000 ppm, using ethyl acetate as the blank. Each concentration was tested in triplicate using a spectrophotometer set at 5 nm intervals within the 290–320 nm range. The obtained absorbance values were applied to the SPF calculation formula to determine the extract's protective capability. The sunscreen potential of *Rhizophora apiculata* extract was assessed based on the resulting SPF values (Morocho-Jácome et al., 2021).

Data analysis

Total phenolic content of *R.apiculata* Extracts

The total phenolic content is calculated by substituting the value of gallic acid in the sample into the following formula (Sanchez et al., 2013):

$$TPC = \frac{V \times C \times DF}{g}$$

note:

TPC	= Phenol content (mgGAE/g)
V	= Volume of sample (mL)
C	= Concentration (mg/mL)
DF	= Dilution factor
g	= Mass of sample (g)

In Vitro SPF Determination

The results of the crude extract analysis were obtained using spectrophotometry, and the absorbance value were calculated using the SPF formula (Hidayah et al., 2023):

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

note:

EE(λ)	= Erythema effect spectrum
I(λ)	= Intensity of spectrum
Abs	= Absorbance of sample
CF	= Correction factor (10)

Result

Phenolic content

The calculation of the regression equation using the gallic acid standard curve yielded a total phenolic value of 63.80 ± 0.12 mg GAE/g for the *R.apiculata* sample.

GC-MS Analysis

The GC-MS chromatogram data of the *R. apiculata* mangrove leaf extract revealed 33 active compounds. Among these, three dominant compounds such as Lupeol, Betulinaldehyde, and Taraxasterol were identified, characterized by the highest chromatogram peaks. The chromatogram of GC-MS of extract can be seen in Figure 1 and its active compound name in Table 1. Below

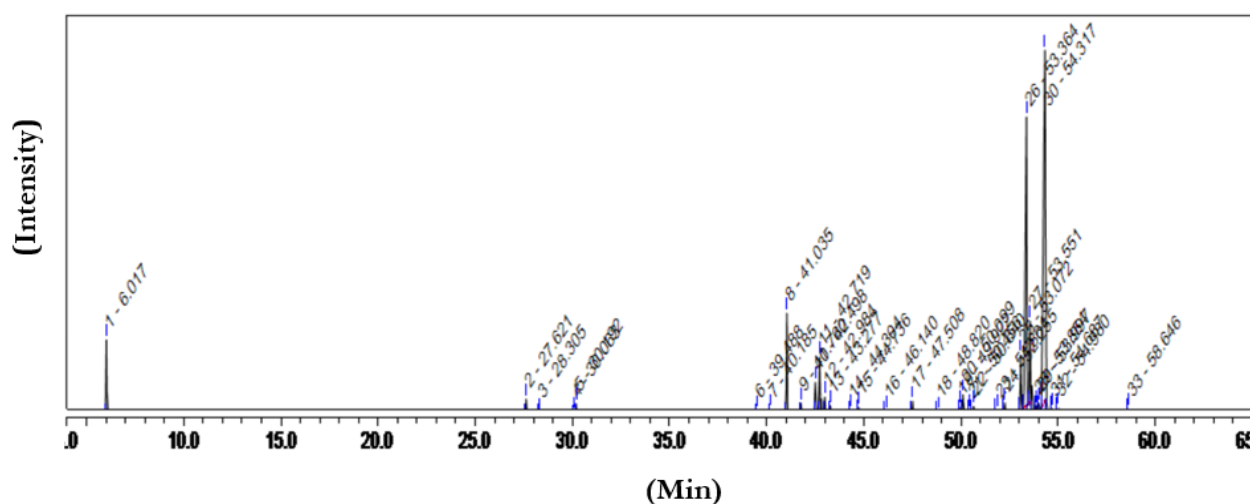


Figure 1. GC-MS chromatogram of the ethyl acetate extract of *R. apiculata***Table 1.** Name of compounds

No	Real Time	Rel. Area (%)	Compounds
1	6,02	3,86	<i>1,3,5,7-Cyclooctatetraene</i>
2	27,62	1,10	<i>Naphthalene, 1,2,3,4-tetrahydro-2-phenyl-</i>
3	28,30	0,35	<i>Cyclobutane, 1,2-diphenyl</i>
4	30,06	0,37	<i>Neophytadiene</i>
5	30,19	0,80	<i>2-Pentadecanone, 6,10,14-trimethyl-</i>
6	39,49	0,44	<i>4,8,12,16-Tetramethylheptadecan-4-olide</i>
7	40,18	0,43	<i>Heptadecane, 2,6,10,15-tetramethyl-</i>
8	41,04	4,90	<i>Cyclohexane, 1,3,5-triphenyl-</i>
9	41,76	0,67	<i>Pentacosane</i>
10	42,50	2,24	<i>(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide</i>
11	42,72	2,59	<i>Benzene, 1,1'-[2(phenylthio)cyclopropylidene]bis-</i>
12	42,98	1,05	<i>Thiocarbamic acid, N,N-dimethyl,S-1,3-diphenyl ester</i>
13	43,28	0,54	<i>Eicosane</i>
14	44,30	0,32	<i>(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide</i>
15	44,74	0,43	<i>2-methylhexacosane</i>
16	46,14	0,35	<i>ter-Hexadecanethiol</i>
17	47,51	0,82	<i>Tritetracontane</i>
18	48,82	0,36	<i>7-Methyl-Z-tetradecen-1-ol acetate</i>
19	49,92	0,53	<i>1-Heptatriacotanol</i>
20	50,10	1,35	<i>Ethanol,2-(octadecyloxy)</i>
21	50,46	0,31	<i>Fumaric acid, 4-octyl dodec-2-en-1-yl ester</i>
22	50,64	0,61	<i>α-Tocopheryl acetate</i>
23	51,86	0,33	<i>Campesterol</i>
24	52,26	0,55	<i>Stigmasterol</i>
25	53,07	3,06	<i>β-Sitosterol</i>
26	53,36	24,76	<i>Friedelan-3-one</i>
27	53,55	4,29	<i>β-Amyrin</i>
28	53,85	0,39	<i>Taraxasterol</i>
29	54,00	0,60	<i>Androst-5-en-3-ol, 4,4-dimethyl-, (3β)-</i>
30	54,32	40,22	<i>Lupeol</i>

31	54,69	0,30	18,19-seculopane-3-ol
32	54,98	0,47	1-Heptatriacotanol
33	58,65	0,59	Betulinaldehyde

Sun Protection Factor (SPF)

SPF assessment was conducted on *Rhizophora apiculata* leaf extracts at various concentrations, including 25, 50, 100, 200, 500, and 1000 ppm. The resulting SPF values are presented in Table 2. Below

Table 2. Sun Protection Factor (SPF)

		Protection (Lionetti & Rigano et al., 2017)
Concentration	SPF	
25	2.530 ± 0.03	Low
50	3.515 ± 0.04	Low
100	6.063 ± 0.07	Low
200	9.464 ± 0.12	Low
500	20.808 ± 0.27	Medium
1000	37.080 ± 0.47	High

Discussion

Total phenolic content

The total phenolic content of *Rhizophora apiculata* leaf extract obtained through maceration with ethyl acetate was 63.80 ± 0.12 mg GAE/g. This value is higher than those reported in other mangrove species, such as *R. stylosa* leaves (9.74 ± 0.75 mg GAE/g) and *Sonneratia alba* leaves (8.27 ± 1.13 mg GAE/g) using methanol as the solvent (Suh et al., 2014), and ethyl acetate leaf extract of *R. mucronata* (23.880±1.099) (Sammanta et al., 2024). Meanwhile, this value is lower than that of the methanolic leaf extract of *Avicennia marina* (148.0 mg GAE/g) (Mitra et al., 2023) and the ethyl acetate leaf extract of *Aegiceras officinalis* (108.16 ± 0.013 mg GAE/g) (108.16±0.013) (Bui et al., 2021). The total phenolic content in plants is influenced by various factors, including the plant species, the specific part of the plant analyzed, and the extraction solvent used (Anokwuru et al., 2011). Mangrove plants often respond to environmental stress through metabolic changes, as evidenced by variations in the production of secondary metabolites, which are predominantly concentrated in their leaves. (Yeshe et al., 2022 and Mitra et al., 2023).

GC-MS profile

GC-MS analysis is a valuable method for identifying various bioactive compounds commonly

used in the food, cosmetics, and pharmaceutical industries (Gomathi et al., 2015). The GC-MS chromatogram of the ethyl acetate extract of *Rhizophora apiculata* [Figure 1] revealed 33 peaks, indicating the presence of approximately 33 bioactive phytocompounds that contribute to the plant's pharmacological activity. The identified compounds, along with their peak number, retention time (RT), and peak area (%), are listed in Table 1. Among the 33 detected peaks, four were particularly prominent. The major compounds identified were lupeol (40.22%), friedelan-3-one (24.76%), cyclohexane-1,3,5-triphenyl (4.9%), and 1,3,5,7-cyclooctatetraene (3.86%).

Lupeol is known for its beneficial biological activities. Several studies have demonstrated that lupeol exhibits antioxidant, anti-inflammatory, and antibacterial properties. In the cosmetics industry, lupeol is recognized as a key ingredient in product formulations (Malinowska et al., 2021). According to Cardoso (2022), compounds such as lupeol, β-sitosterol, stigmasterol, and campesterol can be incorporated as active ingredients in sunscreen formulations due to their protective and therapeutic potential. Sundar (2021) reported that topical creams formulated with a combination of phytochemicals, including lupeol, exhibit potent antioxidant activity and demonstrate significant anticancer potential. Meanwhile, Friedelan-3-one, a pentacyclic triterpene, possesses antioxidant and hepatoprotective properties, and shows potential as a photoprotective agent by reducing UV-induced oxidative stress (Atewolara et al., 2020 and Sunil et al., 2021).

Sunscreen activity

The Sun Protection Factor (SPF) is a key parameter used to evaluate the potential of a substance as a UV filter. Globally, SPF values are considered the gold standard for assessing the efficacy of sunscreen products in protecting the skin from ultraviolet (UV) radiation. This parameter is commonly determined using UV-Visible (UV-Vis) spectrophotometry.

The SPF (Sun Protection Factor) values presented in Table 2 indicate that the ethyl acetate extract of *Rhizophora apiculata* leaves demonstrates potential as a UV filter, with protection levels ranging from low to high protection. The results show that increasing the concentration of the extract correlates positively with

an increase in SPF value. The highest SPF value, 37.080 ± 0.47 , was observed at a concentration of 1000 ppm. In comparison, ethyl acetate extracts of *Avicennia marina* and *Avicennia floridum* have also demonstrated ultra-protection efficacy at a lower concentration (50 ppm), with SPF values of 36 and 39, respectively (Otay et al., 2022). According to (Olayemi et al., 2017) An SPF value above 15 suggests strong potential for a compound to act as an effective sunscreen agent.

Vitamin E (α -tocopherol) has been widely recognized for its antioxidant properties, which contribute to skin protection against oxidative stress (George et al., 2023). According to (Ningrum et al., 2024), further reported that antioxidants play a crucial role in preventing skin damage caused by free radicals generated through UV exposure. Moreover, certain flavonoids exhibit significant antioxidant activity by scavenging reactive oxygen species (ROS) formed during excessive ultraviolet radiation. (Ferreira et al., 2021).

The protective role of antioxidants in preventing photoaging is largely attributed to their ability to inhibit the expression and activity of matrix metalloproteinases (MMPs). These zinc-dependent endopeptidases, predominantly secreted by keratinocytes and dermal fibroblasts, are upregulated in response to external stressors such as pro-inflammatory cytokines, oxidative stress, and ultraviolet (UV) radiation. By suppressing MMP activity, antioxidants contribute to preserving the structural integrity of the skin, minimizing collagen degradation, and reducing the detrimental effects associated with photoaging. (Chen et al., 2012; Madan and Nanda, 2018). According to (Madan and Nanda, 2018) The presence of carboxylic acids, phenolic acids, fatty acids, flavonoids, stilbenoids, and terpenoids contributes to the potential of plant-derived compounds as effective sunscreen agents. Several studies have demonstrated that flavonoids can inhibit the production of hydrogen peroxide induced by ultraviolet (UV) radiation in keratinocytes and reduce malondialdehyde (MDA) levels, a key biomarker of oxidative stress, following sunlight exposure (Kumawat et al., 2018 and Hidayah et al., 2023).

Conclusion

The results revealed that the ethyl acetate extract of *Rhizophora apiculata* mangrove leaves contained a total phenolic content of 63.80 ± 0.12 mg GAE/g. Additionally, the extract exhibited notable photoprotective activity, with the highest SPF value

of 37.080 ± 0.47 recorded at a concentration of 1000 ppm, classifying it as offering high-level protection.

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